

GEL ELECTROPHORESIS OF PCR PRODUCTS

1. Pour a 2% agarose gel using 1X TBE buffer.

- *Mix 5gm of agarose with 250ml of 1X TBE in a 500ml Erlenmyer flask.**
- *Microwave 4 min 20 sec at 50% power. Keep covered and watch for boiling.**
- *Cool to 55° C (10-15 min).**
- *Add 25µl 10mg/ml EtBr.**
- *Pour in casting frame (about 225ml) and remove bubbles.**
- *Place 4 combs in positions 1,2,4, & 6. (2 plates per gel)**

2. Load 4µl of GibcoBRL 100bp ladder (stock is 1µg/µL; dilute with loading buffer to 0.1µg/µL and store frozen) at first and last lane of each row.

3. Use cordless pipettor to pick up 2µL of PCR product, then pick up 6.5µL of loading buffer and load into gel.

4. Run gel at 300 Watts for 25 minutes. or until 1st dark dye front is half-way down the lane

5. Analyze gel image on the digital camera. (Save on zip disk and print a photo).

MULT IMAGE LIGHT CABINET **ALPHA INNOTECH CORP.**

Click and open Alpha Imager 2000 3.3b

Flip on Epi-white light

- with door open place gel in frame (dry off bottom) on glass—center in viewing frame
- adjust (zoom in or out with 2nd dial) until entire gel is in focus, adjust aperture w/ upper dial
- close door and flip off Epi-white light

Turn on UV lamp (button on bottom right)

Click expose (good gel will expose in 8/30 sec. to 12/30 sec.)

Adjust black, white, and gamma for best view desired---click “freeze”

Turn off lamp

Save image on zip disk (drive d)

Print image